Report No. IITRI-L6023-5
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration

IIT RESEARCH INSTITUTE

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February 15 to May 15, 1966

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I. INTRODUCTION

Martian environment simulation experiments are in progress to study the effects of the following barometric pressures and carbon dioxide concentrations on Bacillus cereus spores:

(1) Earth atmosphere at pressures of 10, 25, 40, and 98 mb, with diurnal temperature cycle

(2) Carbon dioxide concentrations of 37, 67, and 100% at pressure of 98 mb, with diurnal temperature cycle

(3) Carbon dioxide concentrations and pressures of 37% at 40 mb, 67% at 25 mb and 100% at 10 mb, with diurnal temperature cycle.

B. cereus spore germination was inhibited by carbon dioxide concentrations of 37, 67, and 100% at all pressures. Pressures as low as 10 mb with Earth atmosphere were not inhibitory to spore germination, but vegetative cell growth was less than that at 98 mb.

Soil ecology experiments were initiated to determine the minimum numbers of a bacterium required to survive and grow in different soil types. A California desert soil with water added to establish an equilibrium relative humidity of 99% is being used. Experiments with B. cereus, Pseudomonas aeruginosa, Putrefactive Anaerobe (PA 3679), and Staphylococcus aureus are in progress.

All organisms, except P. aeruginosa, survived inoculation procedures at 50 to 100% recovery levels; less than 10% of the pseudomonads were recovered. Preliminary results indicated that inocula of P. aeruginosa and S. aureus of 200 to 300 cells per gram of soil do not survive 7 days at either a constant 30°C or a diurnal temperature cycle in the desert soil with 99% relative humidity and 1013 mb Earth atmosphere.

II. EXPERIMENTAL PROCEDURES

P. aeruginosa ATCC 10145 and S. aureus ATCC 12411 were grown on the surface of trypticase soy with 2% agar for 24 hr at 35°C. Maximum spore production was obtained by growing B. cereus on TAM (Difco) for 6 days at 35°C and PA 3679 in a 5.0% trypticase and 0.5% peptone broth for 12 days at 35°C. The cells were harvested and washed with 0.025 M phosphate buffer, pH 7.0, before final suspension in the buffer. The cell suspensions were stored in the refrigerator until used. The *B. cereus* and PA 3679 spore suspensions were heat-shocked at 80°C for 10 min and at 60°C for 15 min, respectively, before use.

The gas mixtures were obtained from the Matheson Company, Inc., Joliet, Illinois. Gas analyses were performed with the following results:

Tank No.	Gas	Amount Required, %	Analysis, %
1	Carbon dioxide	100	>99
	Nitrogen	0	0
	Argon	0	0
	Oxygen	0	< 5 ppm
2	Carbon Dioxide	67	66.2
	Nitrogen	13	12.6
	Argon	20	21.2
	Oxygen	0	5 ppm
3	Carbon dioxide	33	37
	Nitrogen	33.5	26.9
	Argon	33.5	36.1
	Oxygen	0	< 5 ppm

An electric hygrometer-indicator (Hygrodynamics, Inc., Silver Springs, Maryland), model 15-300, was used to determine equilibrium relative himidity (ERH).

California desert soil[1] (No. 68-3, Mecca Hills, California, 0.5 to 6-in. deep) with a high clay content was used. In the

[1]Soil collected and provided by Dr. R. E. Cameron, Jet Propulsion Laboratory, Pasadena, California.

Martian environment simulation studies, mixtures containing different relative amounts of pulverized felsite and limonite were used.

III. RESULTS AND DISCUSSION

A. Martian Environment Simulation Studies

1. Atmospheric Composition and Pressure

Current estimates of the Martian surface pressure range from 5 to 40 mb. Two important factors from a biological point of view are related to pressure -- (1) carbon dioxide abundance and (2) water availability.

With the established carbon dioxide limits of the Martian atmosphere by spectroscopic analysis the relative abundance of carbon dioxide would range from 100 to 33% over the 5- to 40-mb pressure range. Carbon dioxide is regarded as an essential metabolite for bacteria. Normally, it does not appear to be an essential nutrient since most heterotrophic bacteria produce it in metabolism or utilize the small amount present in air. Some species of bacteria require elevated carbon dioxide concentrations for growth, but there is little information on carbon dioxide toxicity for spore germination or vegetative cell growth.

Similarly, water availability is affected by barometric pressure. At 5 mb, water exists as a vapor at temperatures above 0°C, but at 40 mb, water exists as a liquid at temperatures as high as 30°C. With the liquid phase of water limited because of pressure, growth of a microorganism would occur only over very narrow temperature ranges of the diurnal freezing and thawing cycles.

Figures 1 through 4 show the effect of Earth atmosphere (air) at reduced barometric pressures on B. cereus spores. Each tube in the Martian environment simulation studies contained 1 g of felsite/limonite soil, 1% organic medium and 8 to 9% moisture. The tubes were flushed seven times with air before pressure was established and the tubes were sealed. An 8- and a 20-hr diurnal freeze cycle were used with each pressure and atmosphere.

Spore germination and vegetative cell growth, evidenced by marked increase in total count, occurred at 10, 40, and 98 mb and to a less extent at 25 mb during the 8-hr freeze cycle; growth occurred between 3 and 7 days. The 8-hr freeze cycle total count at 7 days was omitted from Figure 2 because of its uncertainty, but values will be obtained at 28 and 56 days.

As shown in Figures 5 through 7, pressures of 10, 25, and 40 mb with respective carbon dioxide concentrations of 100, 67, and 37% inhibited spore germination of B. cereus for at least 28 days during both the 8- and the 20-hr diurnal freeze cycles. A loss of viability did not accompany inhitition of spore germination.

THE EFFECT OF EARTH ATMOSPHERE AT 10mb PRESSURE ON BACILLUS CEREUS SPORES.

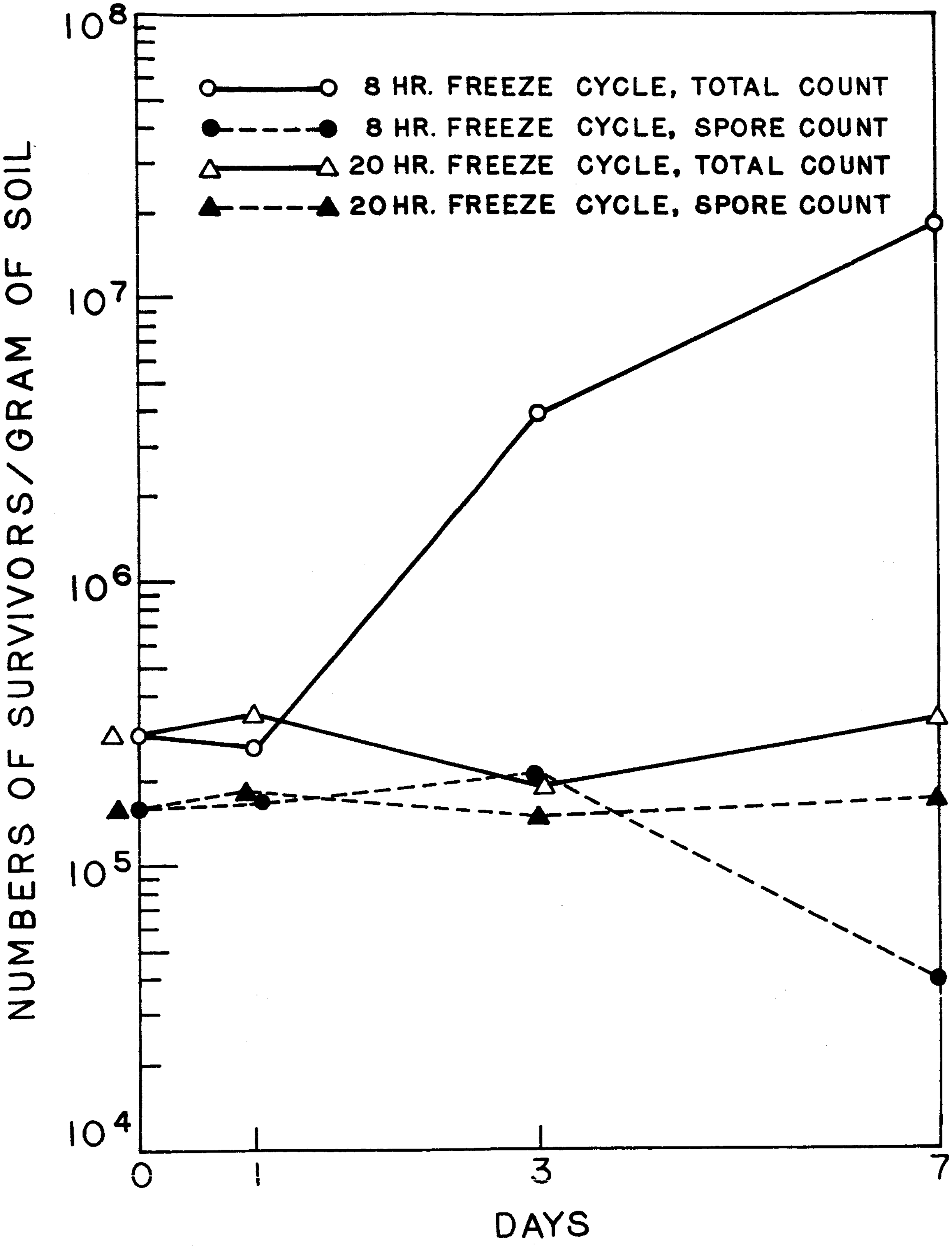

FIGURE 1

THE EFFECT OF EARTH ATMOSPHERE AT 25mb PRESSURE ON *BACILLUS CEREUS* SPORES.

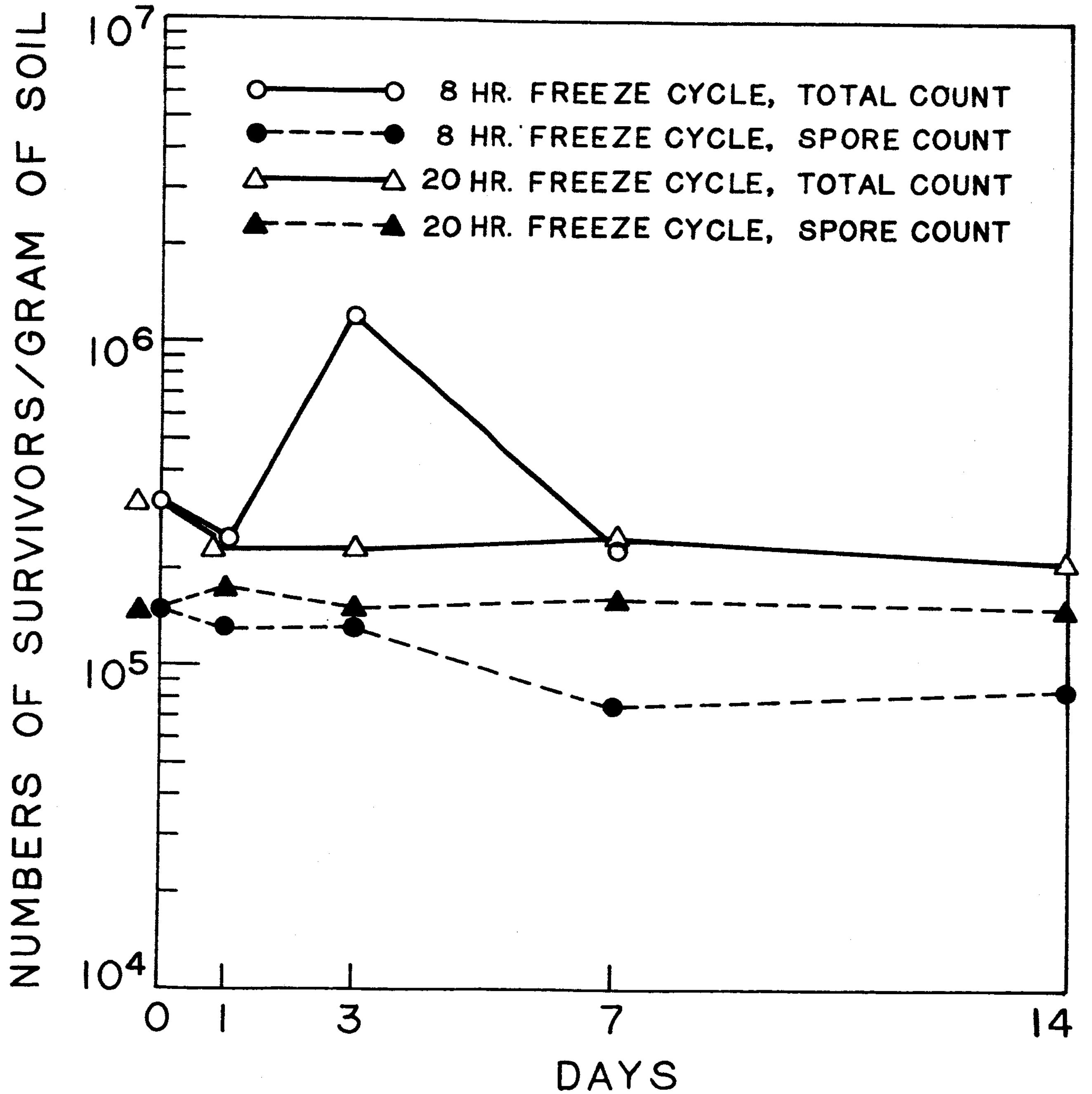

FIGURE 2

THE EFFECT OF EARTH ATMOSPHERE AT 40 mb PRESSURE ON BACILLUS CEREUS SPORES.

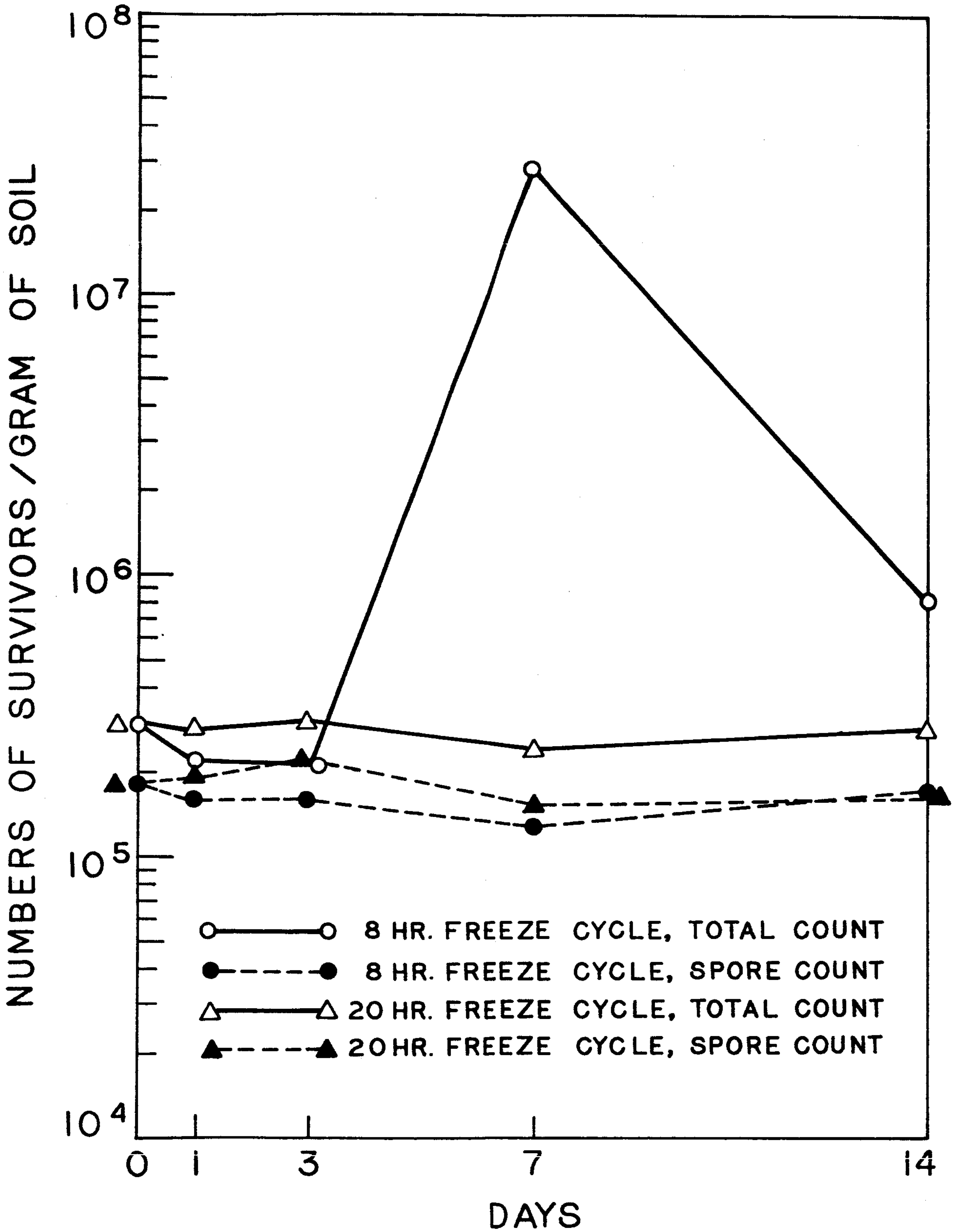

FIGURE 3

THE EFFECT OF EARTH ATMOSPHERE AT 98mb PRESSURE ON BACILLUS CEREUS SPORES.

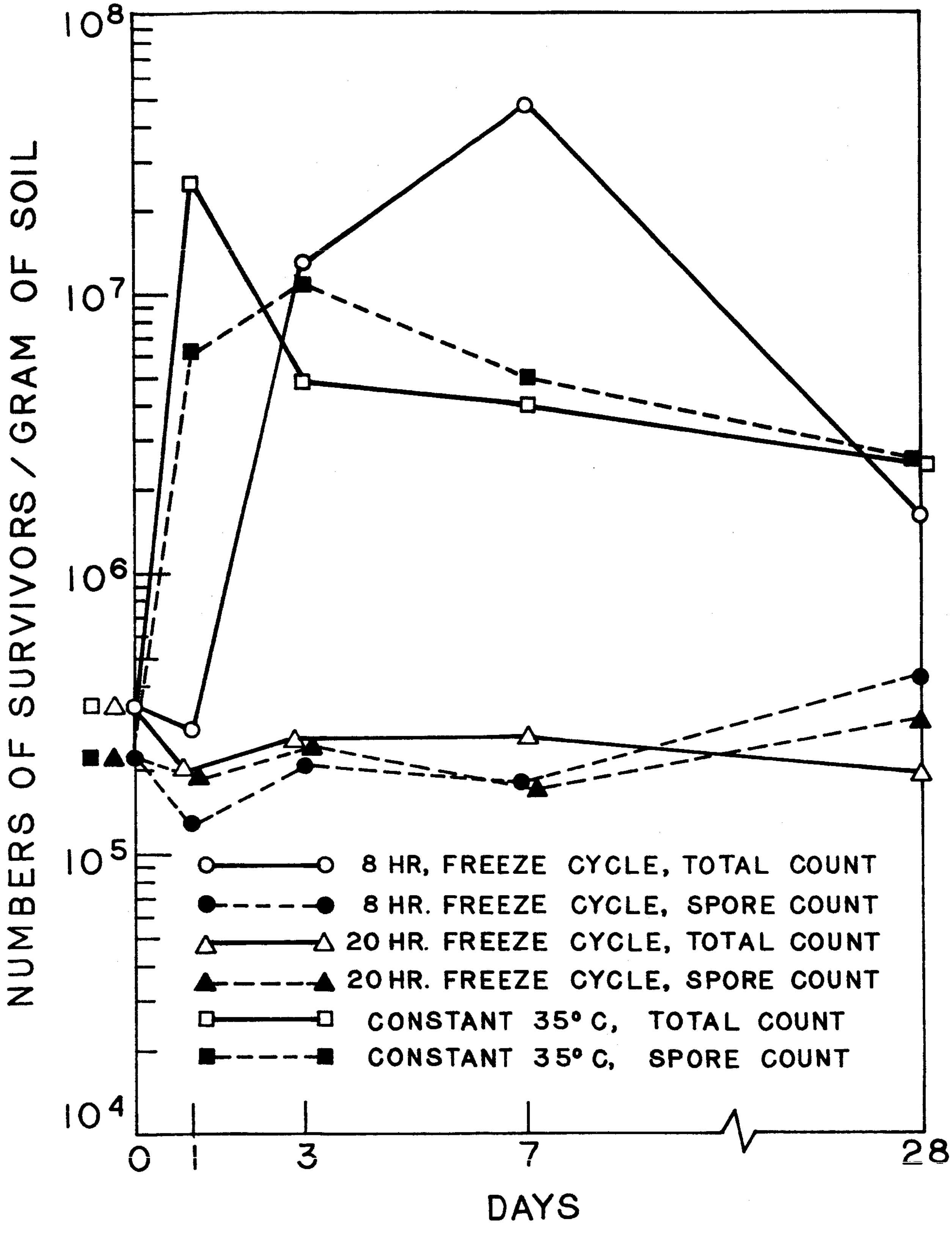

FIGURE 4

THE EFFECT OF 100% CARBON DIOXIDE AT 10mb PRESSURE ON BACILLUS CEREUS SPORES.

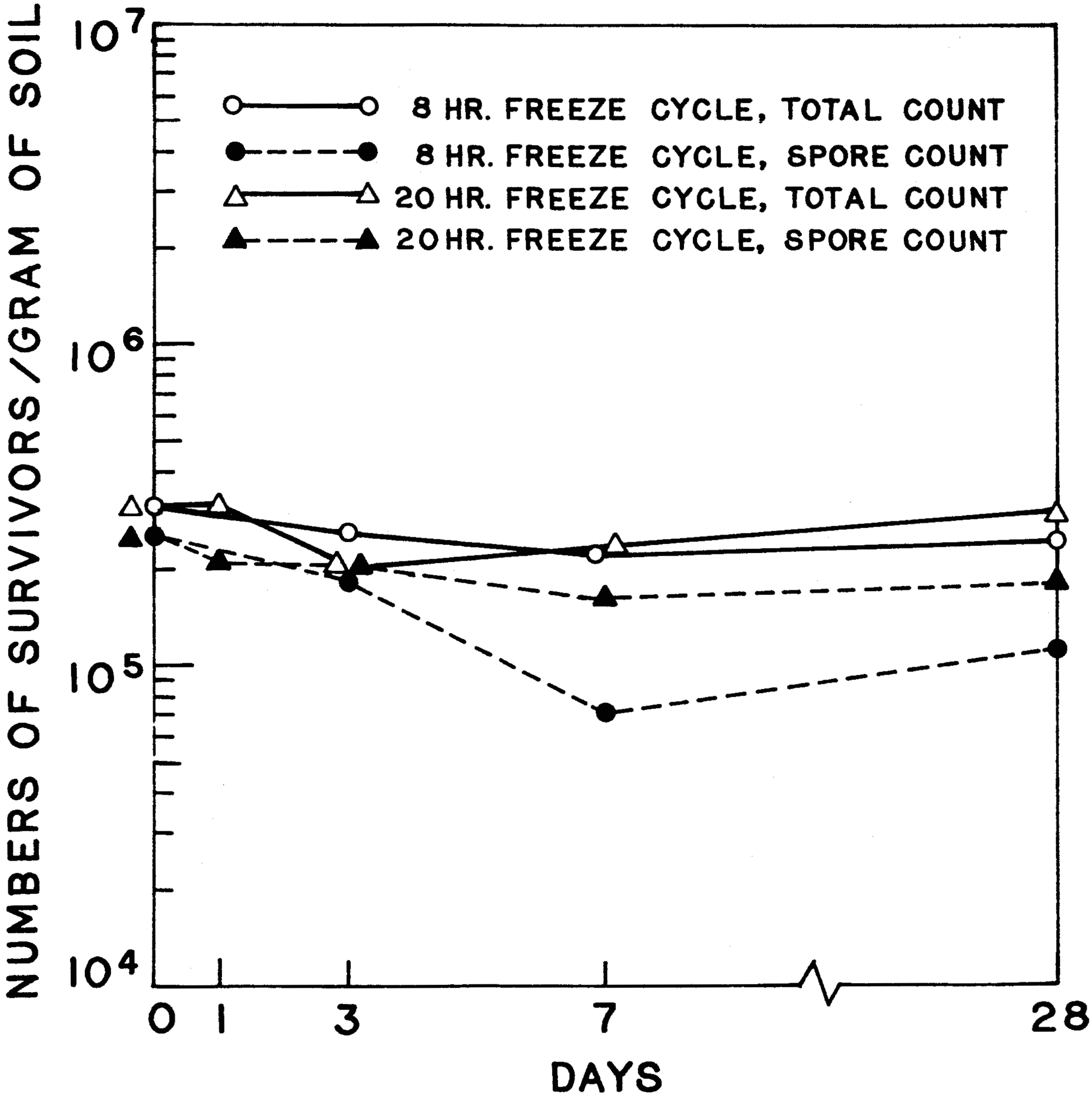

FIGURE 5

THE EFFECT OF 67% CARBON DIOXIDE AT 25mm PRESSURE ON <u>BACILLUS</u> <u>CEREUS</u> SPORES.

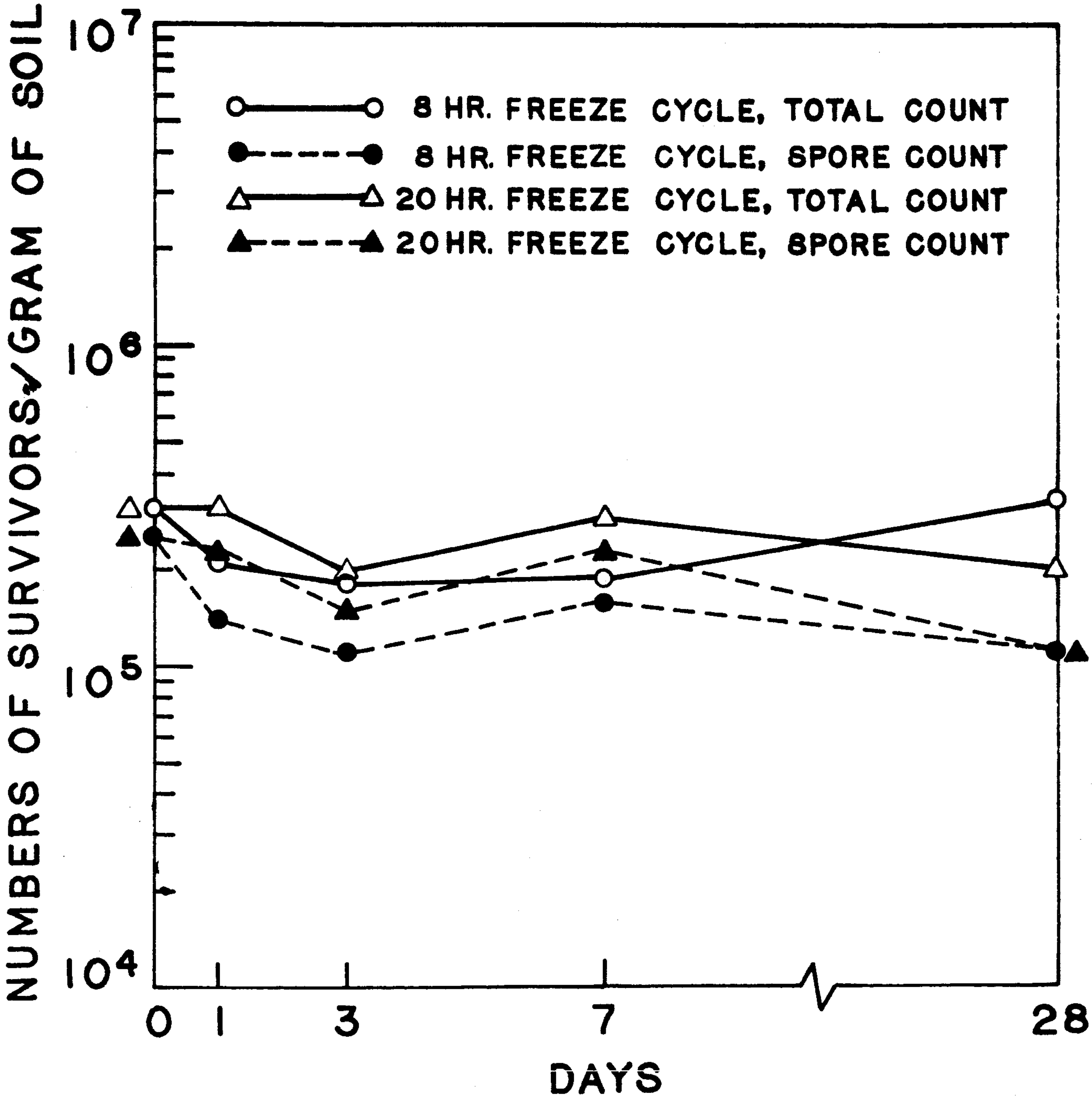

FIGURE 6

THE EFFECT OF 37% CARBON DIOXIDE AT 40mb PRESSURE ON *BACILLUS CEREUS* SPORES.

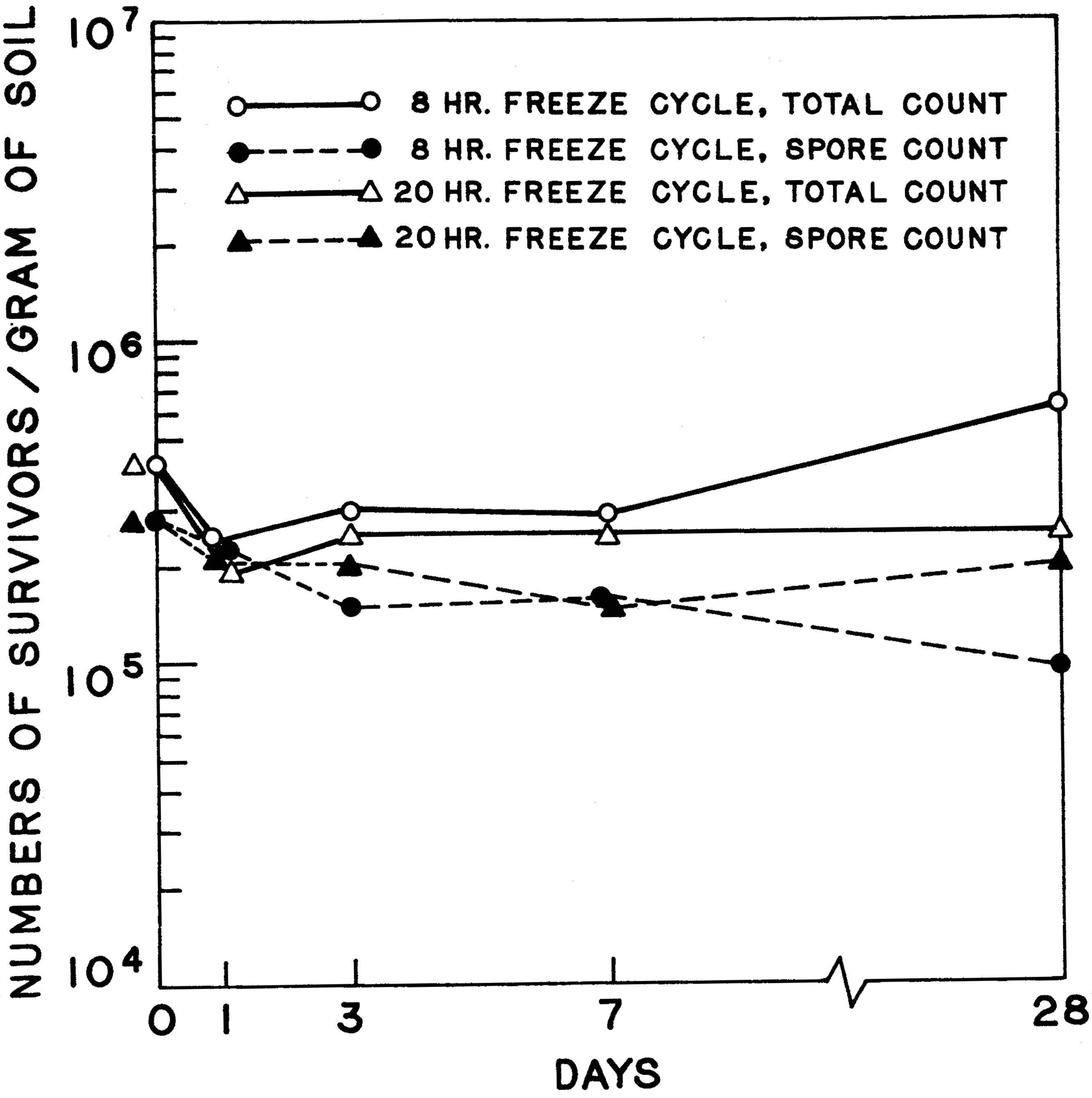

FIGURE 7

Figures 8 and 9 indicate further that inhibition of spore germination was caused by elevated carbon dioxide concentrations. Results from the 100% carbon dioxide experiments are not complete yet, but germination of B. cereus spores was inhibited at both 37 and 67% carbon dioxide levels during the 8- and 20-hr diurnal freeze cycles.

2. Felsite/Limonite Analysis

Inhibition of B. cereus spore germination in some pulverized felsite/limonite mixtures occurred. The pH of the soil is definitely a contributing factor. B. cereus spores in soils of pH 8 to 8.4 do not germinate. Adjustment to pH 7.0 by addition of more limonite permitted spore germination and vegetative cell growth during the 8-hr freeze cycle within 28 to 56 days instead of within the normal 3 to 7 days.

Emission spectroscopy for the following elements did not show any significant differences between the soils: aluminum, arsenic, boron, calcium, chromium, copper, iron, mercury, lithium, magnesium, manganese, sodium, nickel, lead, silicon, tin, titanium, vanadium, and zinc.

Mass spectrometry indicated that of the two soils that inhibited spore germination, one had a high argon content and the other a high sulfur dioxide content. Neither of these substances is of importance because of the heat sterilization and the gassing that the soils receive.

THE EFFECT OF 37% CARBON DIOXIDE AT 98mb PRESSURE ON BACILLUS CEREUS SPORES.

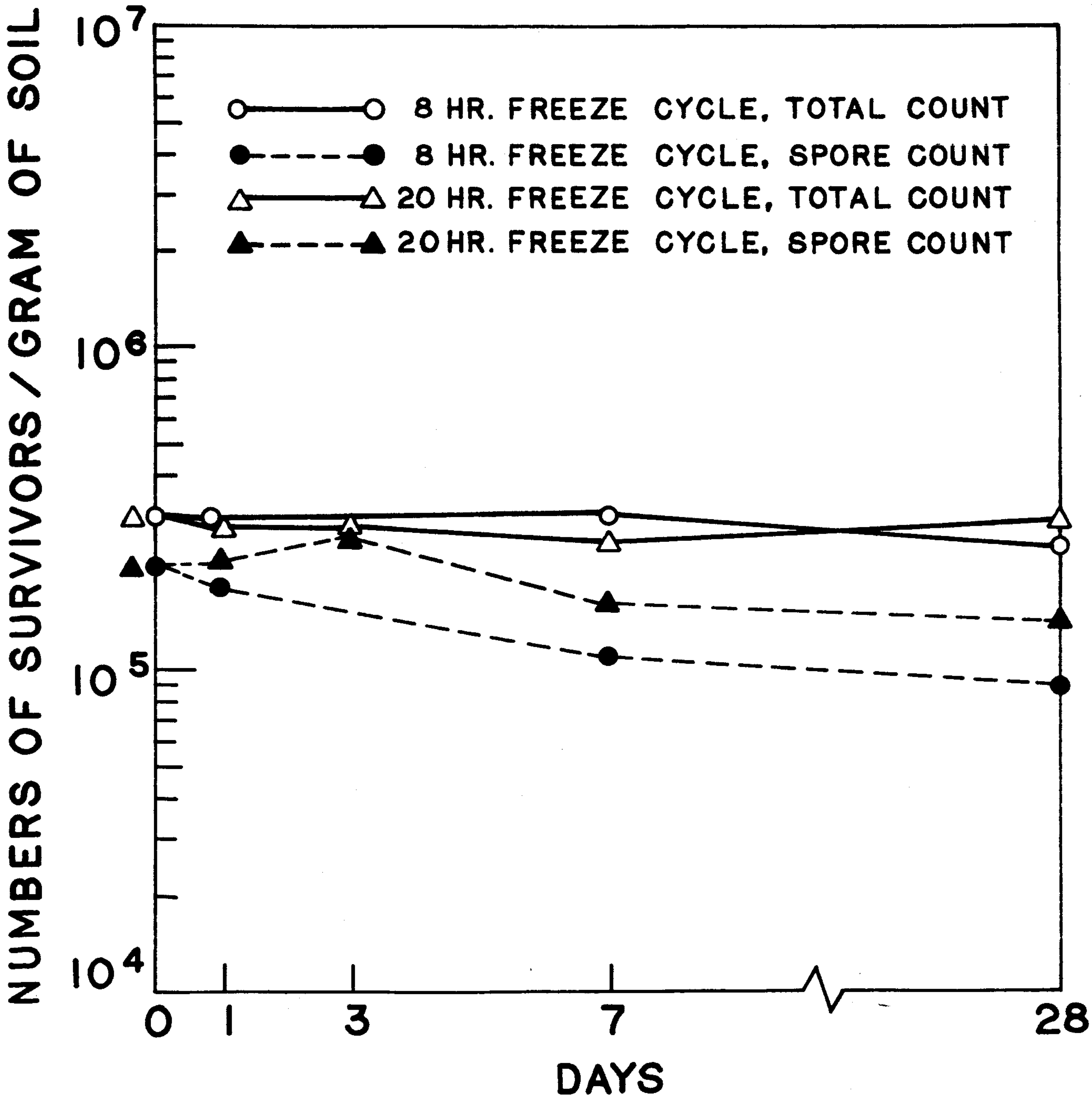

FIGURE 8

THE EFFECT OF 67% CARBON DIOXIDE AT 98mb PRESSURE ON BACILLUS CEREUS SPORES.

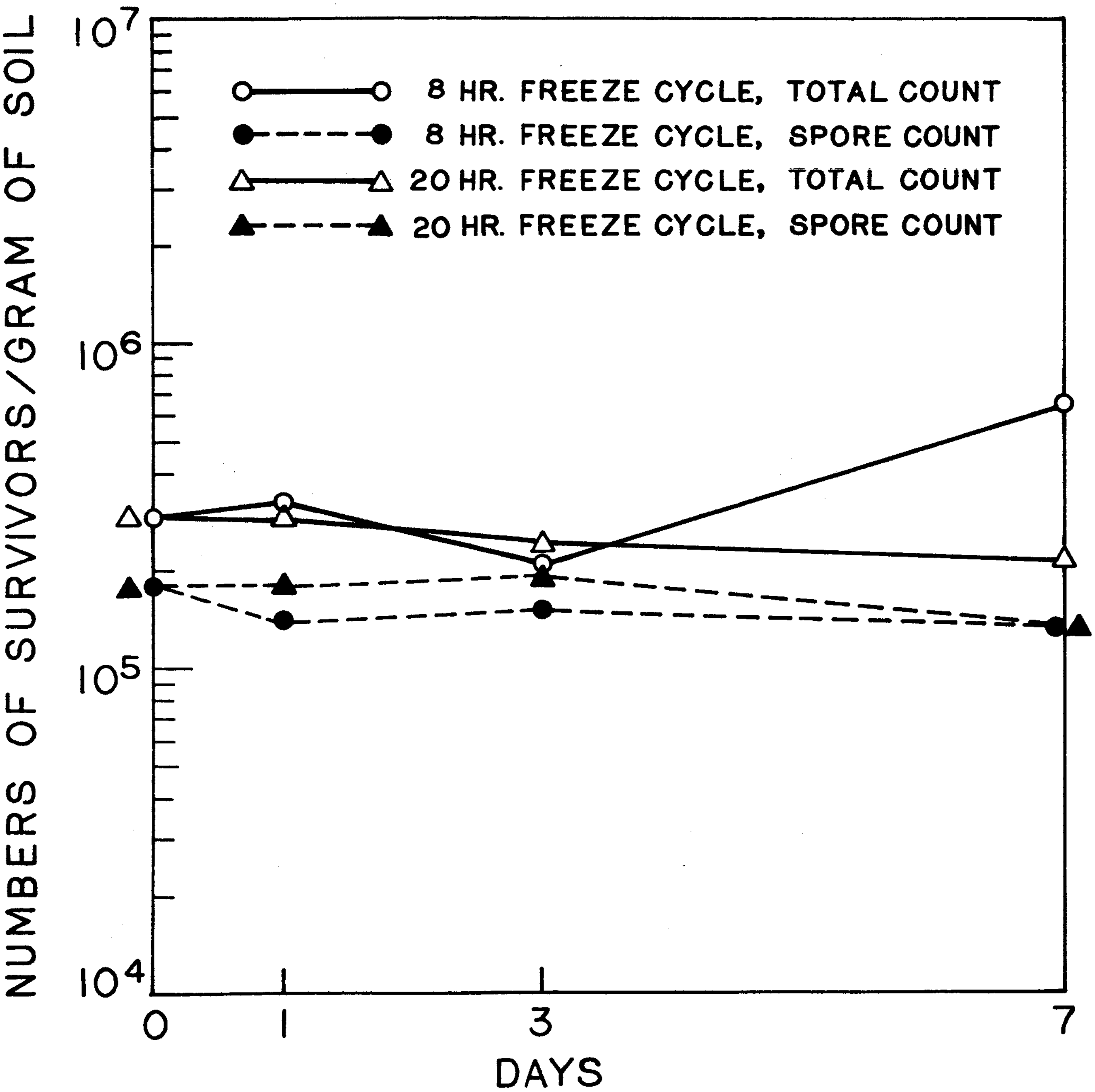

FIGURE 9

Particle sizing of the soils showed that with further addition of pulverized limonite, the percentage of particles less than 44 μ in diameter increased. Soil that permitted normal spore germination and vegetative cell growth had 65% of its particles less than 44 μ in diameter as compared with a poor soil that had 81% of its particles less than 44 μ in diameter.

B. Soil Ecology Studies

Tests were started by using tubes containing 1 g of desert soil, 99% relative humidity, and air at 1013 mb. The tubes were inoculated with diluted culture suspensions of *P. aeruginosa* and *S. aureus* to give 200, 20, 2, and 0.2 cells per gram of soil. The tubes were sealed and placed at either a constant 30°C or a diurnal temperature cycle (-65 to 25°C). At 7 days the tubes were cracked open, and trypticase soy broth was added. The tubes were incubated at 37°C for 7 days, and the contents were streaked on the surface of trypticase soy agar. *P. aeruginosa* and *S. aureus* could not be recovered from any of the tubes.

Another series of experiments with higher cell concentrations are in progress. *P. aeruginosa* was inoculated at 10^3, 10^4, and 10^5 cells per gram of soil, and *B. cereus* was inoculated at 10^2, 10^3, and 10^4 cells per gram of soil. All tubes were placed under the same environmental conditions described above. Preliminary results indicated that 99% of *B. cereus* cells but less than 10% of *P. aeruginosa* cells survived inoculation procedures.

IV. SUMMARY

Carbon dioxide concentrations of 37, 67, and 100% at pressures of 40, 25, and 10 mb, respectively, inhibited B. cereus spore germination during 8- and 20-hr diurnal freeze cycles.

Inhibition of B. cereus spore germination also occurred in atmospheres with 37 and 67% carbon dioxide at 98 mb during both freeze cycles.

B. cereus spore germination was not inhibited in air at reduced pressures of 10, 25, and 40 mb, but the growth response was less than that at 98 mb.

Low numbers of P. aeruginosa and S. aureus did not survive 7 days in a desert soil environment with 99% relative humidity, air at 1013 mb, and either a constant 30°C or a diurnal temperature cycle.

These data will be presented at the forthcoming COSPAR meeting (May 12, 1966), Vienna, Austria. Reprints of the paper will be distributed when available.

V. PERSONNEL AND RECORDS

Experiments were planned with the consel of Dr. E. J. Hawrylewicz and the technical assistance of Miss Marjorie Ewing, Mrs. L. G. Larson, and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks C16678, C16684, C16689, C16876, and C16882.

Respectfully submitted,

IIT RESEARCH INSTITUTE

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CAH/cg

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